Identification of potential key variants in mandibular premolar hypodontia through whole-exome sequencing

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Identification of potential key variants in mandibular premolar hypodontia through whole-exome sequencing

A Dissertation

Submitted to the Department of Dentistry

and the Graduate School of Yonsei University

in partial fulfillment of the

requirements for the degree of

Doctor of Philosophy in Dental Science

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December 2023

This certifies that the Doctoral Dissertation
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December 2023

감사의 글

가장 우선적으로 본 논문이 완성되기까지 항상 아낌없이 따뜻하게 가르쳐주신 이재훈 교수님께 깊은 감사의 마음을 드리고 싶습니다. 또한 본 논문을 위해 귀중한 시간을 내어 꼼꼼하게 살펴봐 주시고 훌륭한 조언과 격려를 해주신 김지환 교수님, 조성원 교수님, 송제선 교수님, 그리고 김상욱 교수님께도 깊은 감사의 말씀을 올립니다. 앞으로도 항상 교수님들께 하나님의 은혜와 축복이 가득하시길 기도드리겠습니다.

다음으로 그 누구보다도 항상 내 편이 되어 응원해주시고 기도해주시는 아버지 어머니, 그 갚지 못할 깊은 사랑을 느끼며 이 자리를 빌려 사랑한다고 전하고 싶습니다. 또 자주보기는 어렵지만 항상 인생에서 롤모델이 되어준 형과 형수님, 그리고 곧 태어날 하늘이에게도 감사의 마음을 전하며, 가까이에서 항상 친부모님처럼 응원해주시고 도와주시는 어머님 아버님께도 감사드립니다. 항상 밝게 맞이해주는 처남과 처남댁, 그리고 너무나도 귀여운 조카 서윤이에게도 고마움을 전하고 싶습니다.

언제나 항상 응원해주고 힘이 되어준 무엇보다도 소중한 우리 아내 지희와 아들 요한이에게 무한한 사랑과 감사를 전하며, 마지막으로 제 인생을 주관하시는 사랑의 하나님께 모든 감사와 영광을 올립니다.

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ABSTRACT

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Determining genotype–phenotype correlations in patients with hypodontia is important for understanding disease pathogenesis, although only a few studies have elucidated it. This study aimed to identify genetic variants linked to non-syndromic bilateral mandibular second premolar hypodontia in a Korean population for the first time by specifying the phenotype of hypodontia.

Twenty unrelated individuals with non-syndromic bilateral mandibular second premolar hypodontia were enrolled for whole-exome sequencing. Using a tooth agenesis gene set panel consisting of 112 genes based on literature, potential candidate variants were screened through variant filtering and prioritization. This study identified 13 candidate variants in 12 genes, including a stop-gain variant (c.4750C>T) in *LAMA3*. Through the functional enrichment analysis

of the prioritized genes, several terms related to tooth development were enriched in a protein–protein interaction network of candidate genes for mandibular premolar hypodontia. The hypodontia group also had approximately 2-fold as many mutated variants in all four genes related to these key terms, which are *CDH1, ITGB4, LAMA3, LAMB3*, as those in the 100 healthy control group individuals. The relationship between enriched terms and pathways and mandibular premolar hypodontia was also investigated. In addition, this study identified some known oligodontia variants in patients with hypodontia, strengthening the possibility of synergistic effects in other genes. This genetic investigation may be a worthwhile preliminary attempt to reveal the pathogenesis of tooth agenesis and sets a background for future studies.

Keywords: Hypodontia, Genotype-phenotype correlation, Genetic association studies, Bioinformatics, Korean population

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I. INTRODUCTION

Congenital tooth agenesis, which is a failure in tooth development, is the most common dental anomaly in humans (De Coster et al., 2009). Tooth agenesis can alter masticatory efficiency, aesthetic appearance, and speech. Studies have found that significant facial changes can occur with congenital tooth agenesis (Taju et al., 2018). Since teeth tend to migrate towards edentulous regions, missing teeth may result in poor positioning of the remaining teeth, making it difficult to manage proper oral rehabilitation. Early diagnosis of tooth agenesis is crucial so that long-term

treatment plans, such as the conservation of deciduous teeth or the maintenance of appropriate space for lost teeth for dental implants, can be established for the patients (Nunn et al., 2003).

Different terms describe tooth agenesis based on the number of missing teeth, excluding third molars. When less than six teeth fail to develop, tooth agenesis is referred to as “hypodontia.” When six or more teeth are missing, tooth agenesis is called “oligodontia,” and “anodontia” refers to the developmental failure of all teeth. Hypodontia is associated with approximately 150 genetic syndromes, including cleft lip and/or cleft palate (Vieira, 2003); however, non-syndromic tooth agenesis (NSTA), in which only dentition is affected as familial or sporadic forms, can also occur without systemic genetic syndromes. Hypodontia can be caused by environmental factors, such as infection, trauma, or early radiation therapy, but is mainly associated with genetic mutations (Ye and Attaie, 2016).

The overall prevalence of hypodontia is 6.4%, but this varies within different continents and racial groups (Khalaf et al., 2014). Mandibular second premolars are the most commonly affected teeth, and there are no specific studies on the prevalence of bilateral mandibular second premolar hypodontia. However, the occurrence can be assumed to be approximately 1% from a meta-analysis report, considering that approximately 21% of tooth agenesis patients have missing bilateral mandibular premolars, and approximately 67% of patients with more than one missing tooth have two missing teeth (Polder et al., 2004).

Teeth develop via a series of complex signaling interactions between the oral epithelium and neural crest-derived mesenchyme through epithelial thickening (initiation) and bud, cap, and bell

stages (Tucker and Sharpe, 2004). These molecular signaling pathways are under strict genetic control during development (Yin and Bian, 2015). Numerous factors, such as nuclear factor (NF)-κB, wingless-related integration site (Wnt), bone morphogenic protein (Bmp), fibroblast growth factor (FGF), and sonic hedgehog (Shh) families, are considerably involved in the regulation of tooth development. Any alteration in these factors can potentially contribute to hypodontia (Galluccio et al., 2012). Since mutations in the *MSX1* and *PAX9* were found to cause NSTA (Stockton et al., 2000; Vastardis et al., 1996), more than 18 genes have been identified as additional causes (Williams and Letra, 2018; AlFawaz et al., 2015; Bonczek et al., 2017). A recent systematic review aimed to investigate the NSTA phenotypes associated with gene mutations and revealed patterns of tooth agenesis based on the identity of mutated genes. For example, mutations in *MSX1* and *PITX2* were reported to be responsible for premolar and third molar agenesis, whereas *PAX9* mutations resulted in agenesis in all molars and mandibular central incisors (Fournier et al., 2018). Two *WNT10A* variants cause maxillary lateral incisor agenesis (Mostowska et al., 2015). In addition, rs15705 and rs3178250 variants of *BMP2* increase the risk of mandibular incisor agenesis (Lu et al., 2016). Despite accumulating evidence for different hypodontia phenotype pathogeneses, research in this field is very limited. Hypodontia shows variable expressivity due to the numerous ways in which 28 unique, permanent teeth could be absent or positioned. Additionally, mutations in several genes contribute to diverse phenotypes with the possibility of oligogenic inheritance. A study that performed genotype–phenotype analysis in patients with *WNT10A* mutations reported that *WNT10A* mutations were associated with the

number and type of tooth agenesis, such as biallelic *WNT10A* mutations associated with absence of maxillary and mandibular molars as well as mandibular central incisors. However, one limitation of this study was the candidate gene analysis (Arzoo et al., 2014).

Recent developments in next-generation sequencing have enabled rapid and efficient analysis of personal DNA genome sequences. Whole-exome sequencing (WES) has received particular attention for the identification of genetic variants of diseases. Exomes refer to all exons within the genome and represent approximately 1.5% of the human genome. WES has been proven effective because up to 90%–85% of known disease-related variants are contained in exons. Earlier, traditional research on non-syndromic hypodontia focused on finding variants in candidate genes, but current trends using WES have revealed new mutated genes for non-syndromic hypodontia (Dinckan et al., 2018; Zhang et al., 2019; Zeng et al., 2017), including *OPN3*, which was previously thought to be irrelevant to tooth development (Inagaki et al., 2021). Genotype–phenotype correlation in hypodontia is important not only for understanding the pathogenesis of the disease but also for the specific tooth regeneration required for replacement eventually (Williams and Letra, 2018; Chhabra et al., 2014). By specifying the phenotype of non-syndromic hypodontia as agenesis of the bilateral mandibular second premolar and applying WES and bioinformatics in the Korean population, this study aimed to better understand the underlying mechanisms of hypodontia by identifying commonalities between hypodontia-causing variants.

II. MATERIALS AND METHODS

1. Participants

Twenty unrelated individuals were included in the study. The participants were selected based on the following criteria: congenital absence of bilateral mandibular second premolars, presence of all other permanent teeth (excluding the third molars), and absence of any other genetic syndromes. They were examined by one dentist for case eligibility using radiographic and clinical examinations and were excluded if they showed any other craniofacial anomaly or syndrome. Eligibility was confirmed after asking participants about their dental and medical histories, including pedigree data, to assess their family history. Panoramic radiographic images, clinical records, and written informed consent were obtained from all patients in this study. One hundred healthy exome sequencing data from 3,703 Ansan–Ansung cohort participants provided by the National Biobank of Korea, the Korea Disease Control and Prevention Agency, Republic of Korea (KBN-2021-063) were used as controls. This clinical study was approved by the Institutional Review Board of the Armed Forces Medical Command (AFMC-20085-IRB-20-085; approved on November 3, 2020). This clinical study was conducted in accordance with the Declaration of Helsinki.

2. DNA sample collection

A 2-mL saliva sample was obtained from each participant using an Oragene DNA self-collection kit (OG-500; DNA Genotek). Each saliva sample was mixed with a solution from the collection kit, which stabilized the sample at room temperature. Genomic DNA was extracted using a preplT-L2P DNA extraction kit (DNA Genotek) according to the manufacturer's instructions. The concentration and purity of the DNA samples were evaluated using agarose gel electrophoresis and a PicoGreen dsDNA Assay (Invitrogen).

3. Whole-exome sequencing (WES)

Briefly, exons were captured according to the manufacturer's instructions using SureSelect Human All Exon V5 (Agilent Technologies), and 2 × 100 base pair reads were sequenced using an Illumina Novaseq 6000 system (Illumina). FASTQC v0.11.5 was used to assess the quality of the read sequences. Each read was compared to the Genome Reference Consortium Human Build 37, and the Burrows–Wheeler Aligner package v0.7.12 was used to align the reads with the reference genome. The reads were realigned around indels, and base quality was recalibrated based on changes using GenomeAnalysisTK (GATK) v3.5. After these steps, alignment quality control was performed, and the variants were called using the GATK HaplotypeCaller gVCF mode. Raw variants were recalibrated using GATK v3.5, and finally, analysis-ready VCF files were produced

using filtered variants.

4. Filtering of exomic variants and data analysis

Two different approaches were applied for the variants' filtering. First, Fisher's exact test was performed to compare the allele frequencies of each variant between the case-control groups. Odds ratios with 95% confidence intervals were used to evaluate the corresponding risks of variants and mandibular premolar hypodontia. PLINK v1.9 was used as the statistical software. Variants with an association $p < 1 \times 10^{-7}$ and odds ratios > 1 were considered statistically significant. Then an additional filter was applied with variants annotated as MODERATE or HIGH impact on SnpEff v4.2 (Cingolani et al., 2012). Finally, variants with a total allele frequency (AF) < 0.01 in the genome aggregation database (gnomAD) v2.1.1 were selected. GnomAD has the largest population variation collection in databases and is used to identify pathogenic variants (Karczewski et al., 2020). Data are available on the gnomAD website (https://gnomad.broadinstitute.org/) (Gudmundsson et al., 2022).

Second, A gene set panel for previously identified genes involved in syndromic tooth agenesis (STA) or NSTA was constructed, consisting of 112 genes from literature (Table 1.) (Yin and Bian, 2015; Williams and Letra, 2018; Andersson et al., 2020). From the genetic variants in the gene set panel, variants annotated as MODERATE or HIGH impact on SnpEff v4.2 with total AFs under 0.01 in both the 1000G project and gnomAD v2.1.1 were selected.

Table 1. A gene-set panel list for previously identified genes identified in syndromic or non-syndromic tooth agenesis obtained from the literature.

Gene name	RefSeq number
ADAMTS2	NM_021599.4
ANKRD11	NM_013275.6
ANTXR1	NM_053034.2
ARL6	NM_177976.3
AXIN2	NM_004655.4
BBS1	NM_024649.5
BCOR	NM_017745.6
CCBE1	NM_133459.4
CCDC28B	NM_024296.5
CDH1	NM_004360.5
CHSY1	NM_014918.5
CLDN1	NM_021101.5
COL17A1	NM_000494.4
COL1A1	NM_000088.4
COL1A2	NM_000089.4
COL3A1	NM_000090.4
COL9A2	NM_001852.4
CREBBP	NM_004380.3
CTSK	NM_000396.4
DUPC1	None
EDA	NM_001399.5
EDA2R	NM_021783.5
EDAR	NM_022336.4
EDARADD	NM_145861.4
ELN	NM_000501.4
EVC	NM_153717.3

EVC2	NM_147127.5
FGD1	NM_004463.3
FGF10	NM_004465.2
FGFR1	NM_023110.3
FGFR2	NM_023029.2
FGFR3	NM_022965.4
FLNA	NM_001456.4
FLNB	NM_001457.4
FOXC1	NM_001453.3
FZR1	NM_016263.4
GJA1	NM_000165.5
GRHL2	NM_024915.4
HOXB1	NM_002144.4
HTNB	None
IFT122	NM_052990.3
IKBKG	NM_003639.4
IRF6	NM_006147.4
IRX5	NM_005853.6
ITGB4	NM_000213.5
JAG1	NM_000214.3
KCNJ2	NM_000891.3
KDM6A	NM_021140.4
KIF1C	NM_006612.6
KISS1R	NM_032551.5
KMT2D	NM_003482.4
KREMEN1	NM_032045.5
LAMA3	NM_198129.4
LAMB3	NM_000228.3
LAMC2	NM_018891.3
MKKS	NM_018848.3

MLXIPL	NM_032954.3
MSX1	NM_002448.3
NEMO	None
NFKBIA	NM_020529.3
NSD1	NM_172349.3
OFD1	NM_003611.3
OTDD	None
P63	None
PAX9	NM_006194.4
PHGDH	NM_006623.4
PIK3R1	NM_181524.2
PITX2	NM_153427.2
PLA2G2A	NM_000300.4
POLR3A	NM_007055.4
POLR3B	NM_018082.6
PORCN	NM_203475.3
PRBNS	None
PRKAR1A	NM_212472.2
PTCH1	NM_000264.5
PTH1R	NM_000316.3
PTHLH	NM_198966.2
PVRL1	None
RBM28	NM_018077.3
RECQL4	NM_004260.4
RMRP	None
ROR2	NM_004560.4
RPS6KA3	NM_004586.3
RSK2	None
SATB2	NM_015265.4
SH3BP2	NM_003023.4

SHH	NM_000193.4
SLC26A2	NM_000112.4
SLC39A13	NM_152264.5
SLC5A5	NM_000453.3
SMOC2	NM_022138.3
SRD5A3	NM_024592.5
TBX1	NM_080647.1
TBX22	NM_016954.2
TBX3	NM_016569.4
TCOF1	NM_000356.4
TFAP2A	NM_001372066.1
TFAP2B	NM_003221.4
TGFA	NM_003236.4
TGFB3	NM_003239.5
TMEM67	NM_153704.6
TP63	NM_003722.5
TRIM37	NM_015294.6
TRISOMY21	None
TSHR	NM_000369.5
TSPEAR	NM_144991.3
TWIST1	NM_000474.4
UBR1	NM_174916.3
VWSM	None
WHSC1	None
WNT10A	NM_025216.3
WNT5A	NM_003392.7

5. Prioritization of mutated variants

To prioritize and elucidate the pathogenicity of mutated variants that may cause mandibular premolar hypodontia, 18 *in silico* prediction tools were applied: Mutation Taster, PROVEAN, SIFT, SIFT4G, Polyphen2, MetaSVM, REVEL, LIST-S2, LRT, M-CAP, Mutation Assessor, BayesDel noAF, MetaLR, DANN, EIGEN, EIGEN PC, FATHMM, and FATHMM-MKL. SnpEff was used to identify missense variants from SIFT and PolyPhen2. In addition, the scores of Combined Annotation-Dependent Depletion (CADD) were checked for candidate variants. CADD integrates multiple annotations, such as sequence conservation score and ENCODE project functional annotations, and expresses the variants' deleteriousness as a number (Kircher et al., 2014). The CADD score can be considered a ranking, and the higher the score, the more deleterious the variant. Variants that were estimated as deleterious by at least half of the *in silico* prediction tools (more than medium in the case of Mutation Assessor) and had CADD scores above 20 were prioritized. Prioritized variants were compared with the Human Gene Mutation Database to check if they were previously reported in tooth agenesis. Prioritized variants were finally ruled out if they were classified as benign using the ClinVar database or the American College of Medical Genetics and Genomics (ACMG) guidelines. According to a recent report, Sanger sequencing validation was not performed, considering this study only detected single nucleotide variants, and the coverage depth of WES obviated the need (Arteche-López et al., 2021).

6. *In silico* functional analysis of candidate genes related to the disease

The computational tool STRING v11.5 (Szklarczyk et al., 2019) was applied to examine the pathways and functional terms associated with the candidate genes of mandibular premolar hypodontia. 12 candidate genes obtained from WES were inputted with “homo sapiens″ as the organism. The multiple proteins query was used and “full STRING network,” “medium confidence level,” and “medium false discovery rate (FDR) stringency.” were selected. A protein–protein interaction (PPI) network was visualized, and functional enrichment analysis was performed in the network. The following categories were analyzed for functional enrichment using Fisher’s exact test with Benjamini–Hochberg FDR correction: Gene Ontology (GO), STRING Clusters, KEGG Pathways, Reactome Pathways, Wiki Pathways, Disease-gene associations (DISEASES), Subcellular localization (COMPARTMENTS), Human Phenotype (Monarch), UniProt Annotated Keywords, and SMART protein domains. To compare the total number of mutations in the candidate genes between the case-control groups, the alternate/reference allele ratio of all variants’ sites was calculated. Both synonymous and non-synonymous variants were obtained and odds ratios with p-values were performed by Fisher's exact test and Bonferroni's correction.

III. RESULTS

1. Patient characteristics

The 20 samples for WES analysis comprised 18 males and 2 females. The median age of the participants in the sample was 21 years (range: 19–65 years). After clinical and radiographic evaluations, all patients were diagnosed with non-syndromic bilateral mandibular second premolar hypodontia (Figure 1). No other signs of genetic syndromes were found in any of the patients.

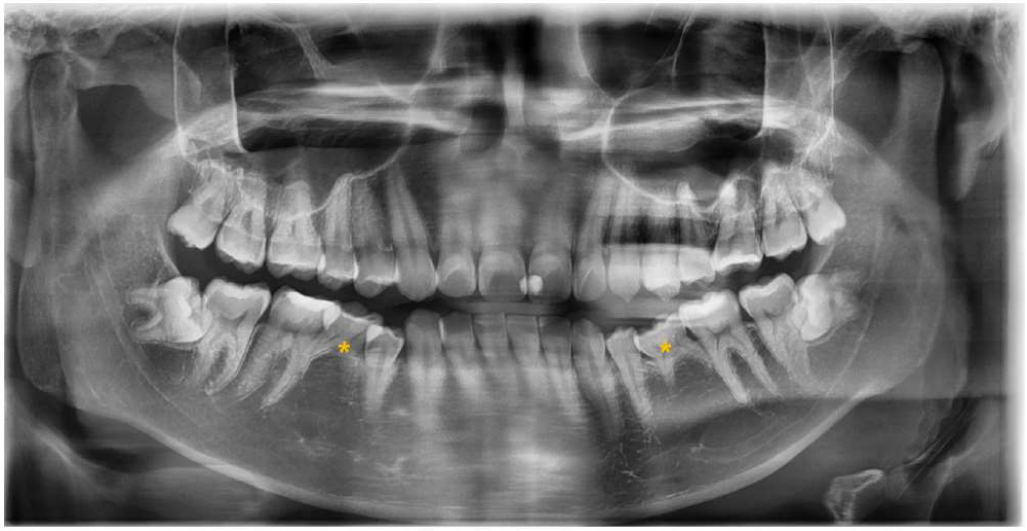

Figure 1. Panoramic radiograph of the subject with a congenital lack of mandibular second premolars (indicated with asterisks).

2. Candidate variants found in WES analysis and prioritization

A total of 112,487 variants were found in the 20 samples after performing WES. Of these, 853 variants were considered statistically significant after the case-control group comparison, but none met the prioritization criteria. From the gene set panel, 13 variants in 12 genes were found in 11 patients. Of these, 12 variants were missense, and 1 variant was stop-gain, and all 13 variants were heterozygous. No variant was identified in the tooth agenesis gene set panel from the other nine patients. These variants were divided into three categories: known variants, candidate variants in NSTA genes, and candidate variants in STA genes.

3. Known variants

Two variants from three patients were identified from samples previously reported in oligodontia patients (Table 2). A known missense variant (c.1138A>C) in *EDAR* was found in two patients (Zeng et al., 2017). This variant has been reported pathogenic for non-syndromic oligodontia but benign for ectodermal dysplasia in the ClinVar database. Another known missense variant (c.511C>T) in *WNT10A* was also found in these samples (Zeng et al., 2017; Song et al., 2014; Zhao et al., 2019; Park et al., 2019; Zhou et al., 2021; Xie et al., 2022).

Table 2. List of known variants.

			Variant				*In silico* pathogenicity prediction																	CADD
Patient no.	Effect	Gene name	HGVS.c	HGVS.p	Transcripts	AF	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	score
1	missense	*EDAR*	c.1138A>C	p.Ser380Arg	NM_022336.4	0.001	DC	N	D	D	PD	D	LDC	D	D		N	D	T	U	U	U	D	D	28
2	missense	*EDAR*	c.1138A>C	p.Ser380Arg	NM_022336.4	0.001	DC	N	D	D	PD	D	LDC	D	D		N	D	T	U	U	U	D	D	28
3	missense	*WNT10A*	c.511C>T	p.Arg171Cys	NM_025216.3	0.001	DC	D	D	D	PD	T	LDC	D	N		M	T	T	U	U	U	T	D	24.8

HGVS.c, coding variant; HGVS.p, protein-level variant; Transcripts, mRNA transcript number; AF, allele frequency from gnomAD v.2 database; *in silico* prediction tools (1-Mutation Taster, 2-PROVEAN, 3-SIFT, 4-SIFT4G, 5-Polyphen2, 6-MetaSVM, 7-REVEL, 8-LIST-S2, 9-LRT, 10-M-CAP, 11-Mutation Assessor, 12-BayesDel noAF, 13-MetaLR, 14-DANN, 15-EIGEN, 16-EIGEN PC, 17-FATHMM, and 18-FATHMM-MKL); DC, disease causing; D, deleterious/damaging; LDC, likely disease causing; PD, probably damaging; U, uncertain significance; T, tolerated; N, neutral; M, medium); CADD, Combined Annotation-Dependent Depletion

4. Candidate variants in NSTA genes

Five candidate variants were identified in the five NSTA genes (Table 3). A missense variant (c.697G>A) in *WNT10A* has not been previously reported, but a different nucleotide change in the same codon (c.697G>T) was identified in a previous study (Dinckan et al., 2018). No variant was found in *MSX1, PAX9, AXIN2, PITX2, EDA*, or *EDARRD*.

Table 3. List of candidate variants in NSTA genes.

		Variant					*In silico* pathogenicity prediction																					CADD
Patient no.	Effect	Gene name	HGVS.c	HGVS.p	Transcripts	AF	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	score
4	missense	*WNT10A*	c.697G>A	p.Glu233Lys	NM_025216.3	0.00007	D	C	N	D	D	P	D	D	L	D	C	D	D	D	M	D	D	U	P	P	T	D	29.3
5	missense	*FGFR1*	c.2294G>A	p.Arg765Gln	NM_023110.3	0.00002	D	C	D	D	D	P	D	T	L	D	C	D	D	D	N	D	T	P	U	U	D	D	27.6
6	stop gained	*LAMA3*	c.4750C>T	p.Arg1584*	NM_198129.4	0.0001																							36
7	missense	*KREMEN1*	c.196C>A	p.His66Asn	NM_001039570.3	0.0005	D	C	D	D	D	P	D	T	L	B	D	D	D	M	T	T	U	P	P	T	D		24.8
8	missense	*SMOC2*	c.947C>T	p.Pro316Leu	NM_001166412.2	0.0002	D	C	D	D	D	P	D	T	L	B	D	D	D	M	T	T	U	U	U	T	D		25.6

HGVS.c, coding variant; HGVS.p, protein level variant; Transcripts, mRNA transcript number; AF, allele frequency from gnomAD v.2 database; *in silico* prediction tools (1-Mutation Taster, 2-PROVEAN, 3-SIFT, 4-SIFT4G, 5-Polyphen2, 6-MetaSVM, 7-REVEL, 8-LIST-S2, 9-LRT, 10-M-CAP, 11-Mutation Assessor, 12-BayesDel noAF, 13-MetaLR, 14-DANN, 15-EIGEN, 16-EIGEN PC, 17-FATHMM, and 18-FATHMM-MKL); DC, disease causing; D, deleterious/damaging; LDC, likely disease causing; PD, probably damaging; U, uncertain significance; T, tolerated; N, neutral; L, low; M, medium; CADD, Combined Annotation-Dependent Depletion; *, stop gained codon

5. Candidate variants in STA genes

Six variants in six STA genes were identified from the samples (Table 4). Patient 9 had a missense variant (c.3052G>A) in *FLNB* with a missense variant (c.329C>A) in *BBS1*. The *FLNB* variant has conflicting interpretations as three submissions as uncertain significance and one as benign, and the *BBS1* variant was of uncertain significance in the ClinVar database. *FLNB* mutation caused abnormal cranium morphology in a mouse model (Lu et al., 2007). Patient 11 had a missense variant (c.2494G>A) in *CDH1*. A member of the cadherin superfamily, *CDH1* is a transmembrane adhesion protein (Taneyhill, 2008). By regulating cell–cell adhesion or interacting with Wnt intracellular signaling, cadherins serve critical roles in craniofacial morphogenesis and dental development (Schambony et al., 2004; Bienz, 2005; Brembeck et al., 2006; Di Benedetto et al., 2015).

Table 4. List of candidate variants in STA genes.

Patient no.	Variant						*In silico* pathogenicity prediction														CADD score
	Effect	Gene name	HGVS.c	HGVS.p	Transcripts	AF	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	
3	missense	*ITGB4*	c.4564C>T	p.Arg1522Cys	NM_000213.5	0.00002	D	C	D	D	D	P	D	T	U	D	D	D	M	D	T	B	B	B	T	D	24.2
4	missense	*EVC2*	c.1516G>A	p.Glu506Lys	NM_147127.5	0.000003	D	C	N	T	D	P	D	D	L	D	C	D	N	D	M	D	D	U	U	U	T	D	25.4
9	missense	*FLNB*	c.3052G>A	p.Val1018Met	NM_001457.4	0.0002	D	C	N	T	D	P	D	D	L	D	C	D	D	D	M	D	D	U	U	U	D	D	23.8
9	missense	*BBS1*	c.329C>A	p.Pro110His	NM_024649.5	0.00001	D	C	D	D	D	P	D	D	L	D	C	D		D	M	D	D	U	P	P	D	D	25
10	missense	*LAMB3*	c.734G>A	p.Arg245His	NM_000228.3	0.0003	D	C	N	D	D	P	D	T	L	B		D	D	M	T	T	P	U	B	T	N		23.9
11	missense	*CDH1*	c.2494G>A	p.Val832Met	NM_004360.5	0.0001	D	C	N	D	D	P	D	D	L	D	C	D	D	D	M	D	D	U	P	P	T	D	28.7

HGVS.c, coding variant; HGVS.p, protein level variant; Transcripts, mRNA transcript number; AF, allele frequency from the gnomAD v.2 database; *in silico* prediction tools (1-Mutation Taster, 2-PROVEAN, 3-SIFT, 4-SIFT4G, 5-Polyphen2, 6-MetaSVM, 7-REVEL, 8-LIST-S2, 9-LRT, 10-M-CAP, 11-Mutation Assessor, 12-BayesDel noAF, 13-MetaLR, 14-DANN, 15-EIGEN, 16-EIGEN PC, 17-FATHMM, and 18-FATHMM-MKL); DC, disease-causing; D, deleterious/damaging; LDC, likely disease-causing; PD, probably damaging; U, uncertain significance; B, benign; T, tolerated; N, neutral; M, medium; CADD, Combined Annotation-Dependent Depletion

6. Predicted effect of a stop-gain variant in *LAMA3*

A stop-gain variant (c.4750C>T) in *LAMA3* found in patient 6 was focused on. The transcript domain was located in the laminin IV type A domain, causing termination at the protein position 1,584 instead of 3,333 (Figure 2). This variant can lead to functional consequences due to production of a truncated protein or degradation of the transcript by Nonsense-Mediated Decay (NMD). Mutated LAMA3 protein loses coiled-coil and globular laminin domains, which have adhesion, signaling, differentiation, and gene expression functions. The CADD score was very high at 36, and the variant's clinical significance was conflicting interpretations of pathogenicity with two uncertain significances for junctional epidermolysis bullosa and one likely benign in the ClinVar database (accession number VCV000551372.4).

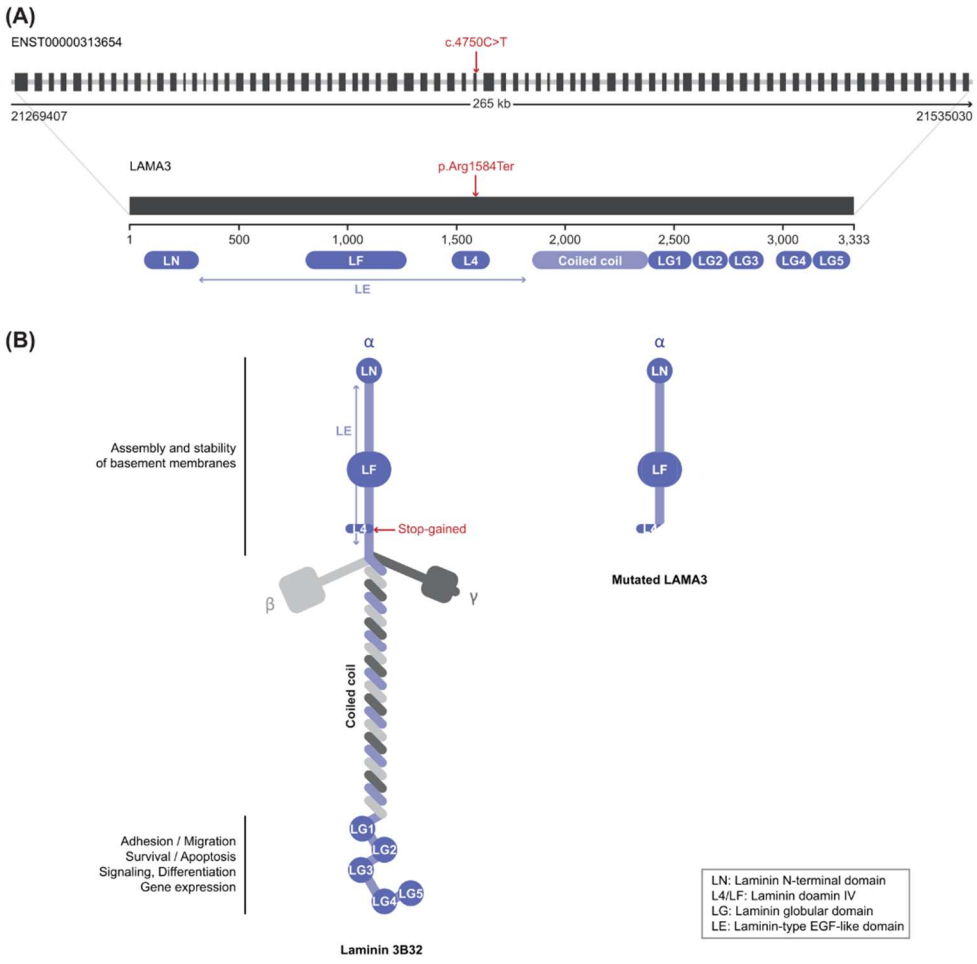

Figure 2. Schematic view of the LAMA3 structure. (A) Introns are presented as gray lines and coding exons as black boxes (upper structure). Variants identified in this study are indicated by red characters. (B) Native (left) and mutated (right) LAMA3 protein structures. Each domain's name and role are explained in the figure.

7. *In silico* functional study of the candidate genes

The PPI network for candidate genes for mandibular premolar hypodontia was visualized using STRING (Figure 3). In total, 343 terms were functionally enriched. Of these, 13 were enriched in GO, 8 in KEGG Pathways, 9 in Reactome Pathways, 7 in Wiki Pathways, 12 in DISEASES, 1 in COMPARTMENTS, 280 in Monarch, 12 in UniProt Annotated Keywords, and 1 in SMART. The terms were mainly related to “cell junction,” “extracellular matrix,” “laminin,” and “basement membrane.” The key terms in the PPI network were color-coded, and these are elaborated on in Table 5. Eight out of the 12 candidate genes were found to be significant in case-control comparisons of alternate/reference allele ratio. In particular, hypodontia groups had mutated variants approximately 2-fold as much in all four genes related to “cell junction assembly” and “extracellular matrix organization” (Table 6). Whole functional enrichment analysis results are listed on Table 7.

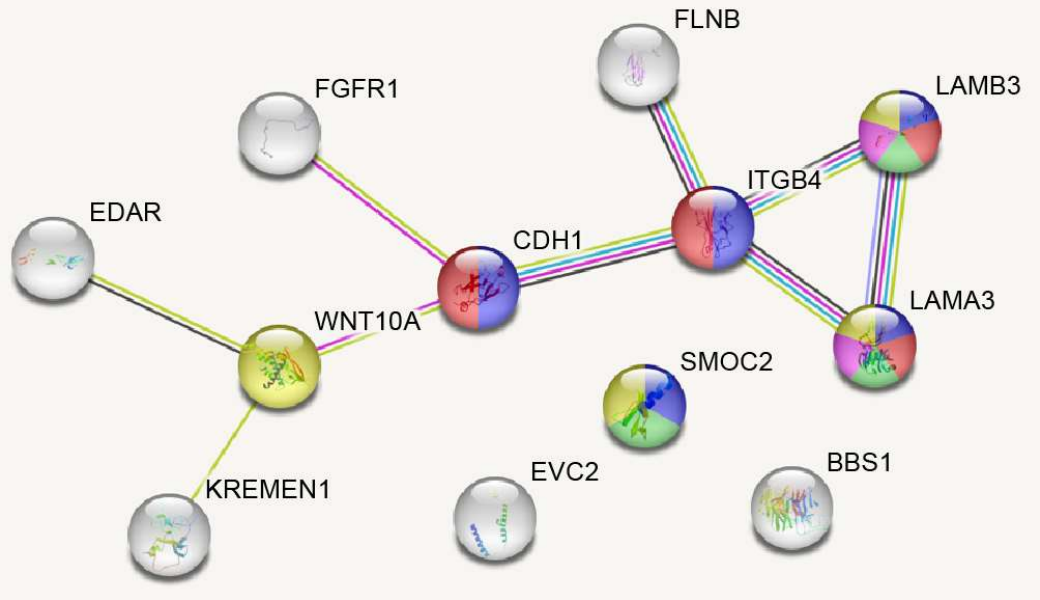

Figure 3. The protein–protein interaction network of variants found in mandibular premolar hypodontia predicted by STRING. Network nodes and edges represent proteins and protein–protein associations, respectively.

Table 5. Key functional enrichment analysis result terms.

Color	Category	Term ID	Term description	Count	Background	Strength	FDR	Gene
red	GO Process	GO:0034329	Cell junction assembly	4	280	1.37	0.028	*ITGB4,CDH1,LAMA3,LAMB3*
blue	GO Process	GO:0030198	Extracellular matrix organization	5	338	1.38	0.0049	*ITGB4,CDH1,LAMA3, SMOC2,LAMB3*
green	GO Component	GO:0005604	Basement membrane	3	96	1.71	0.0227	*LAMA3,SMOC2,LAMB3*
magenta	COMPARTMENTS	GOCC:0005610	laminin-5 complex	2	7	2.67	0.0286	*LAMA3,LAMB3*
yellow	UniProt Keywords	KW-0272	Extracellular matrix	4	265	1.39	0.0013	*WNT10A,LAMA3,SMOC2,LAMB3*

The colors are those from Figure 3. FDR; *p*-value after false discovery rate correction

Table 6. Fisher's exact test results of the alternate/reference allele ratio of candidate genes related to key functional terms between the case-control groups.

Gene	Odds ratio	*p*-value	Case allele count		Control allele count	
			Ref	Alt	Ref	Alt
CDH1	2.092093	1.35E-44	2199	721	15703	2461
ITGB4	2.004838	1.81E-178	6911	3129	70734	15974
LAMA3	1.868188	1.55E-85	6048	1712	56560	8570
LAMB3	1.574748	7.63E-30	2524	1356	19589	6683

Table 7. Functional enrichment analysis results of candidate genes for mandibular premolar hypodontia

Category	Term ID	Term description	Count	Background	Strength	FDR	Matching proteins in the network
GO Process	GO:0008544	Epidermis development	6	419	1.37	0.001	WNT10A,EDAR,LAMA3,LAMB3,FGFR1,FLNB
GO Process	GO:0031581	Hemidesmosome assembly	3	12	2.61	0.001	ITGB4,LAMA3,LAMB3
GO Process	GO:0030198	Extracellular matrix organization	5	338	1.38	0.0049	ITGB4,CDH1,LAMA3,SMOC2,LAMB3
GO Process	GO:0009653	Anatomical structure morphogenesis	8	2165	0.78	0.0137	ITGB4,WNT10A,EDAR,CDH1,LAMA3,LAMB3,FGFR1,FLNB
GO Process	GO:0009887	Animal organ morphogenesis	6	967	1.01	0.017	ITGB4,WNT10A,EDAR,LAMA3,LAMB3,FGFR1
GO Process	GO:0034329	Cell junction assembly	4	280	1.37	0.028	ITGB4,CDH1,LAMA3,LAMB3
GO Process	GO:0009888	Tissue development	7	1760	0.81	0.0326	ITGB4,WNT10A,EDAR,LAMA3,LAMB3,FGFR1,FLNB
GO Process	GO:0030154	Cell differentiation	9	3702	0.6	0.0461	ITGB4,WNT10A,EDAR,C

							DH1,LAMA3,KREMEN1,LAMB3,FGFR1,FLNB
GO Process	GO:0001704	Formation of primary germ layer	3	116	1.62	0.0478	ITGB4,LAMA3,LAMB3
GO Process	GO:2001028	Positive regulation of endothelial cell chemotaxis	2	15	2.34	0.0478	SMOC2,FGFR1
GO Function	GO:0050839	Cell adhesion molecule binding	5	538	1.18	0.0351	ITGB4,CDH1,LAMA3,LAMB3,FLNB
GO Component	GO:0005610	laminin-5 complex	2	3	3.04	0.0058	LAMA3,LAMB3
GO Component	GO:0005604	Basement membrane	3	96	1.71	0.0227	LAMA3,SMOC2,LAMB3
KEGG	hsa04510	Focal adhesion	4	198	1.52	0.0017	ITGB4,LAMA3,LAMB3,FLNB
KEGG	hsa05200	Pathways in cancer	5	517	1.2	0.0017	WNT10A,CDH1,LAMA3,LAMB3,FGFR1
KEGG	hsa04512	ECM-receptor interaction	3	88	1.74	0.0023	ITGB4,LAMA3,LAMB3
KEGG	hsa05165	Human papillomavirus	4	325	1.3	0.0029	ITGB4,WNT10A,LAMA3,L

		infection					AMB3
KEGG	hsa04151	PI3K-Akt signaling pathway	4	350	1.27	0.0031	ITGB4,LAMA3,LAMB3,FGFR1
KEGG	hsa05205	Proteoglycans in cancer	3	196	1.4	0.0119	WNT10A,FGFR1,FLNB
KEGG	hsa04520	Adherens junction	2	67	1.69	0.0379	CDH1,FGFR1
KEGG	hsa05218	Melanoma	2	72	1.66	0.0381	CDH1,FGFR1
Reactome	HSA-446107	Type I hemidesmosome assembly	3	11	2.65	0.00014	ITGB4,LAMA3,LAMB3
Reactome	HSA-446728	Cell junction organization	4	92	1.85	0.00028	ITGB4,CDH1,LAMA3,LAMB3
Reactome	HSA-3000157	Laminin interactions	3	30	2.21	0.00069	ITGB4,LAMA3,LAMB3
Reactome	HSA-2022090	Assembly of collagen fibrils and other multimeric structures	3	61	1.9	0.0028	ITGB4,LAMA3,LAMB3
Reactome	HSA-3000171	Non-integrin membrane-ECM interactions	3	59	1.92	0.0028	ITGB4,LAMA3,LAMB3
Reactome	HSA-1474244	Extracellular matrix organization	4	301	1.34	0.007	ITGB4,CDH1,LAMA3,LAMB3
Reactome	HSA-	Anchoring fibril	2	15	2.34	0.0112	LAMA3,LAMB3

	2214320	formation					
Reactome	HSA-1474228	Degradation of the extracellular matrix	3	140	1.54	0.0174	CDH1,LAMA3,LAMB3
Reactome	HSA-8874081	MET activates PTK2 signaling	2	30	2.04	0.0334	LAMA3,LAMB3
WikiPathways	WP244	Alpha 6 beta 4 signaling pathway	3	33	2.17	0.00084	ITGB4,LAMA3,LAMB3
WikiPathways	WP306	Focal adhesion	4	196	1.52	0.0017	ITGB4,LAMA3,LAMB3,FLNB
WikiPathways	WP3932	Focal adhesion: PI3K-Akt-mTOR-signaling pathway	4	302	1.33	0.0059	ITGB4,LAMA3,LAMB3,FGFR1
WikiPathways	WP4172	PI3K-Akt signaling pathway	4	336	1.29	0.0067	ITGB4,LAMA3,LAMB3,FGFR1
WikiPathways	WP4541	Hippo-Merlin signaling dysregulation	3	119	1.61	0.0068	ITGB4,CDH1,FGFR1
WikiPathways	WP2839	Hair follicle development: organogenesis - part 2 of 3	2	32	2.01	0.0216	EDAR,CDH1

WikiPathways	WP4534	Mechanoregulation and pathology of YAP/TAZ via Hippo and non-Hippo mechanisms	2	46	1.85	0.037	ITGB4,CDH1
Monarch	HP:0009804	Tooth agenesis	11	274	1.82	6.03E-16	ITGB4,WNT10A,EDAR,CDH1,BBS1,LAMA3,KREMEN1,EVC2,LAMB3,FGFR1,FLNB
Monarch	HP:0000164	Abnormality of the dentition	12	822	1.38	1.64E-13	ITGB4,WNT10A,EDAR,CDH1,BBS1,LAMA3,KREMEN1,EVC2,SMOC2,LAMB3,FGFR1,FLNB
Monarch	HP:0000668	Hypodontia	9	160	1.96	1.64E-13	ITGB4,WNT10A,EDAR,CDH1,BBS1,LAMA3,EVC2,LAMB3,FLNB
Monarch	HP:0011061	Abnormality of dental structure	9	238	1.79	3.69E-12	ITGB4,WNT10A,EDAR,CDH1,LAMA3,SMOC2,LAMB3,FGFR1,FLNB
Monarch	HP:0008386	Aplasia/Hypoplasia of the nails	8	191	1.83	9.38E-11	ITGB4,WNT10A,CDH1,LAMA3,EVC2,LAMB3,FGFR

							1,FLNB
Monarch	HP:0000685	Hypoplasia of teeth	7	101	2.05	1.63E-10	ITGB4,WNT10A,EDAR,LAMA3,LAMB3,FGFR1,FLNB
Monarch	HP:0001597	Abnormality of the nail	9	393	1.57	1.75E-10	ITGB4,WNT10A,EDAR,CDH1,LAMA3,EVC2,LAMB3,FGFR1,FLNB
Monarch	HP:0002231	Sparse body hair	6	56	2.24	8.88E-10	ITGB4,WNT10A,EDAR,LAMA3,LAMB3,FGFR1
Monarch	HP:0011362	Abnormal hair quantity	9	508	1.46	1.32E-09	ITGB4,WNT10A,EDAR,CDH1,LAMA3,KREMEN1,EVC2,LAMB3,FGFR1
Monarch	HP:0008070	Sparse hair	8	300	1.64	1.45E-09	ITGB4,WNT10A,EDAR,CDH1,LAMA3,KREMEN1,LAMB3,FGFR1
Monarch	HP:0006297	Enamel hypoplasia	6	82	2.08	5.18E-09	ITGB4,WNT10A,LAMA3,LAMB3,FGFR1,FLNB
Monarch	HP:0006482	Abnormality of dental morphology	7	228	1.7	1.88E-08	WNT10A,EDAR,CDH1,EVC2,SMOC2,LAMB3,FGFR1
Monarch	HP:0000691	Microdontia	6	110	1.95	2.41E-	WNT10A,EDAR,EVC2,SM

						08	OC2,LAMB3,FGFR1
Monarch	HP:0002164	Nail dysplasia	6	145	1.83	0.0000 00084	ITGB4,WNT10A,EDAR,LAMA3,EVC2,LAMB3
Monarch	EFO:0000508	Genetic disorder	7	332	1.54	0.0000 00152	ITGB4,CDH1,BBS1,LAMA3,LAMB3,FGFR1,FLNB
Monarch	HP:0006089	Palmar hyperhidrosis	4	17	2.58	0.0000 00217	ITGB4,WNT10A,LAMA3,LAMB3
Monarch	HP:0001574	Abnormality of the integument	11	2269	0.9	0.0000 00232	ITGB4,WNT10A,EDAR,CDH1,BBS1,LAMA3,KREMEN1,EVC2,LAMB3,FGFR1,FLNB
Monarch	HP:0001596	Alopecia	6	187	1.72	0.0000 00296	ITGB4,WNT10A,EDAR,LAMA3,LAMB3,FGFR1
Monarch	HP:0000924	Abnormality of the skeletal system	11	2708	0.82	0.0000 0146	ITGB4,WNT10A,EDAR,CDH1,BBS1,LAMA3,EVC2,SMOC2,LAMB3,FGFR1,FLNB
Monarch	HP:0000492	Abnormal eyelid morphology	7	501	1.36	0.0000 0198	ITGB4,WNT10A,EDAR,CDH1,KREMEN1,FGFR1,FLNB

Monarch	HP:0008404	Nail dystrophy	5	115	1.85	0.00000216	ITGB4,WNT10A,LAMA3,EVC2,LAMB3
Monarch	HP:0000078	Abnormality of the genital system	9	1355	1.03	0.00000234	ITGB4,WNT10A,CDH1,BBS1,LAMA3,EVC2,LAMB3,FGFR1,FLNB
Monarch	HP:0001155	Abnormality of the hand	8	908	1.16	0.00000301	ITGB4,WNT10A,BBS1,LAMA3,EVC2,LAMB3,FGFR1,FLNB
Monarch	HP:0008388	Abnormal toenail morphology	5	125	1.81	0.00000301	ITGB4,WNT10A,EDAR,EVC2,FGFR1
Monarch	HP:0000315	Abnormality of the orbital region	8	923	1.15	0.00000328	ITGB4,WNT10A,EDAR,CDH1,LAMA3,KREMEN1,FGFR1,FLNB
Monarch	HP:0000698	Conical tooth	4	42	2.19	0.00000391	WNT10A,CDH1,EVC2,FGFR1
Monarch	HP:0000679	Taurodontia	4	44	2.17	0.00000452	WNT10A,SMOC2,LAMB3,FGFR1
Monarch	HP:0100871	Abnormality of the palm	6	318	1.49	0.00000459	ITGB4,WNT10A,LAMA3,EVC2,LAMB3,FGFR1
Monarch	HP:0005922	Abnormal hand	7	608	1.27	0.0000	ITGB4,BBS1,LAMA3,EVC

		morphology				0533	2,LAMB3,FGFR1,FLNB
Monarch	HP:0020117	Hypoplastic dermoepidermal hemidesmosomes	3	7	2.84	0.00000533	ITGB4,LAMA3,LAMB3
Monarch	HP:0000499	Abnormal eyelash morphology	5	154	1.72	0.00000579	WNT10A,EDAR,CDH1,KREMEN1,FGFR1
Monarch	HP:0000163	Abnormal oral cavity morphology	7	644	1.25	0.00000652	ITGB4,WNT10A,EDAR,LAMA3,EVC2,LAMB3,FGFR1
Monarch	HP:0007383	Congenital localized absence of skin	3	8	2.79	0.00000652	ITGB4,LAMA3,LAMB3
Monarch	HP:0031446	Erosion of oral mucosa	3	8	2.79	0.00000652	ITGB4,LAMA3,LAMB3
Monarch	HP:0003341	Subepidermal blistering with cleavage in the lamina lucida	3	9	2.74	0.00000788	ITGB4,LAMA3,LAMB3
Monarch	HP:0004057	Mitten deformity	3	9	2.74	0.00000788	ITGB4,LAMA3,LAMB3
Monarch	HP:0011121	Abnormality of skin morphology	9	1649	0.95	0.00000788	ITGB4,WNT10A,EDAR,CDH1,LAMA3,EVC2,LAMB

							3,FGFR1,FLNB
Monarch	HP:0001798	Anonychia	4	59	2.04	0.00000868	ITGB4,WNT10A,LAMA3,LAMB3
Monarch	HP:0006101	Finger syndactyly	5	178	1.66	0.00000904	ITGB4,CDH1,BBS1,FGFR1,FLNB
Monarch	HP:0010476	Aplasia/Hypoplasia of the bladder	3	11	2.65	0.00000958	ITGB4,LAMA3,LAMB3
Monarch	HP:0200097	Oral mucosal blisters	3	11	2.65	0.00000958	ITGB4,LAMA3,LAMB3
Monarch	HP:0009810	Abnormality of upper limb joint	6	421	1.37	0.000125	ITGB4,LAMA3,EVC2,LAMB3,FGFR1,FLNB
Monarch	HP:0001760	Abnormal foot morphology	8	1254	1.02	0.000162	ITGB4,WNT10A,BBS1,LAMA3,EVC2,LAMB3,FGFR1,FLNB
Monarch	HP:0001602	Laryngeal stenosis	3	14	2.54	0.00000166	LAMA3,LAMB3,FLNB
Monarch	HP:0004552	Scarring alopecia of scalp	3	14	2.54	0.00000166	ITGB4,LAMA3,LAMB3
Monarch	HP:0011297	Abnormal digit morphology	8	1263	1.01	0.000166	ITGB4,CDH1,BBS1,LAMA3,EVC2,LAMB3,FGFR1,FL

							NB
Monarch	HP:0012227	Urethral stricture	3	14	2.54	0.0000166	ITGB4,LAMA3,LAMB3
Monarch	HP:0012210	Abnormal renal morphology	7	836	1.14	0.000022	ITGB4,BBS1,LAMA3,EVC2,LAMB3,FGFR1,FLNB
Monarch	HP:0012718	Morphological abnormality of the gastrointestinal tract	7	841	1.13	0.000226	ITGB4,CDH1,BBS1,LAMA3,LAMB3,FGFR1,FLNB
Monarch	HP:0040064	Abnormality of limbs	9	2020	0.86	0.000273	ITGB4,WNT10A,CDH1,BBS1,LAMA3,EVC2,LAMB3,FGFR1,FLNB
Monarch	HP:0000478	Abnormality of the eye	10	2876	0.75	0.000286	ITGB4,WNT10A,CDH1,BBS1,LAMA3,KREMEN1,EVC2,LAMB3,FGFR1,FLNB
Monarch	HP:0005918	Abnormal finger phalanx morphology	6	515	1.28	0.000332	ITGB4,LAMA3,EVC2,LAMB3,FGFR1,FLNB
Monarch	HP:0001056	Milia	3	20	2.39	0.000365	ITGB4,LAMA3,LAMB3
Monarch	HP:0011355	Localized skin lesion	6	527	1.27	0.000367	ITGB4,EDAR,CDH1,LAMA3,LAMB3,FGFR1

Monarch	HP:0200041	Skin erosion	3	21	2.37	0.0000398	ITGB4,LAMA3,LAMB3
Monarch	HP:0011065	Conical incisor	3	23	2.33	0.00005	WNT10A,EVC2,FGFR1
Monarch	HP:0000070	Ureterocele	3	24	2.31	0.00005	ITGB4,LAMA3,LAMB3
Monarch	HP:0001167	Abnormality of finger	7	979	1.07	0.00005	ITGB4,BBS1,LAMA3,EVC2,LAMB3,FGFR1,FLNB
Monarch	EFO:0000408	Disease	8	1529	0.93	0.0000556	ITGB4,CDH1,BBS1,LAMA3,KREMEN1,LAMB3,FGFR1,FLNB
Monarch	HP:0000968	Ectodermal dysplasia	3	28	2.24	0.0000792	WNT10A,EDAR,EVC2
Monarch	HP:0001000	Abnormality of skin pigmentation	5	316	1.41	0.0000797	ITGB4,EDAR,LAMA3,LAMB3,FGFR1
Monarch	HP:0001057	Aplasia cutis congenita	3	30	2.21	0.0000897	ITGB4,LAMA3,LAMB3
Monarch	HP:0000670	Carious teeth	4	130	1.7	0.0000922	ITGB4,CDH1,LAMA3,LAMB3
Monarch	HP:0001808	Fragile nails	3	31	2.2	0.0000	ITGB4,LAMA3,LAMB3

						956	
Monarch	HP:0200035	Skin plaque	3	31	2.2	0.0000	ITGB4,LAMA3,LAMB3
						956	
Monarch	HP:0000137	Abnormality of the ovary	4	141	1.67	0.0001	WNT10A,CDH1,BBS1,FGF
						2	R1
Monarch	HP:0001030	Fragile skin	3	34	2.16	0.0001	ITGB4,LAMA3,LAMB3
						2	
Monarch	HP:0004370	Abnormality of	5	349	1.37	0.0001	ITGB4,EDAR,LAMA3,LA
		temperature regulation				2	MB3,FGFR1
Monarch	HP:0009702	Carpal synostosis	3	34	2.16	0.0001	EVC2,FGFR1,FLNB
						2	
Monarch	HP:0000204	Cleft upper lip	4	144	1.66	0.0001	CDH1,EVC2,FGFR1,FLNB
						3	
Monarch	HP:0000982	Palmoplantar	4	147	1.65	0.0001	ITGB4,WNT10A,LAMA3,L
		keratoderma				3	AMB3
Monarch	HP:0001075	Atrophic scars	3	35	2.15	0.0001	ITGB4,LAMA3,LAMB3
						3	
Monarch	HP:0011368	Epidermal thickening	5	357	1.36	0.0001	ITGB4,WNT10A,LAMA3,L
						3	AMB3,FGFR1
Monarch	HP:0008065	Aplasia/Hypoplasia of	4	149	1.64	0.0001	ITGB4,EDAR,LAMA3,LA

		the skin				4	MB3
Monarch	HP:0011842	Abnormal skeletal morphology	9	2573	0.76	0.0001 5	ITGB4,EDAR,CDH1,BBS1, LAMA3,EVC2,LAMB3,FG FR1,FLNB
Monarch	HP:0001231	Abnormal fingernail morphology	4	157	1.62	0.0001 6	ITGB4,WNT10A,EDAR,EV C2
Monarch	HP:0002031	Abnormal esophagus morphology	4	154	1.63	0.0001 6	ITGB4,LAMA3,LAMB3,F GFR1
Monarch	HP:0006703	Aplasia/Hypoplasia of the lungs	3	39	2.1	0.0001 6	EVC2,FGFR1,FLNB
Monarch	HP:0011793	Neoplasm by anatomical site	6	730	1.13	0.0001 7	WNT10A,CDH1,LAMA3,E VC2,LAMB3,FGFR1
Monarch	HP:0000008	Abnormal morphology of female internal genitalia	5	393	1.32	0.0001 8	WNT10A,CDH1,BBS1,EV C2,FGFR1
Monarch	HP:0008069	Neoplasm of the skin	4	172	1.58	0.0002 2	WNT10A,LAMA3,LAMB3, FGFR1
Monarch	HP:0000126	Hydronephrosis	4	176	1.57	0.0002 4	ITGB4,LAMA3,LAMB3,F GFR1
Monarch	HP:0003549	Abnormality of	7	1294	0.95	0.0002	ITGB4,WNT10A,LAMA3,S

		connective tissue				4	MOC2,LAMB3,FGFR1,FLNB
Monarch	HP:0002577	Abnormal stomach morphology	4	182	1.55	0.00026	ITGB4,CDH1,LAMA3,LAMB3
Monarch	HP:0000677	Oligodontia	3	53	1.97	0.00033	WNT10A,KREMEN1,FGFR1
Monarch	HP:0011830	Abnormal oral mucosa morphology	4	195	1.52	0.00033	ITGB4,LAMA3,EVC2,LAMB3
Monarch	HP:0045005	Neural tube defect	4	204	1.5	0.00039	ITGB4,CDH1,FGFR1,FLNB
Monarch	HP:0002860	Squamous cell carcinoma	3	57	1.93	0.0004	WNT10A,LAMA3,LAMB3
Monarch	HP:0006481	Abnormality of primary teeth	3	58	1.93	0.00042	WNT10A,EDAR,FGFR1
Monarch	HP:0006292	Abnormality of dental eruption	4	212	1.49	0.00044	WNT10A,EVC2,FGFR1,FLNB
Monarch	HP:0000999	Pyoderma	2	5	2.81	0.00047	LAMA3,LAMB3
Monarch	HP:0001829	Foot polydactyly	3	61	1.9	0.00047	BBS1,EVC2,FGFR1

Monarch	HP:0008682	Renal tubular epithelial necrosis	2	5	2.81	0.00047	LAMA3,LAMB3
Monarch	HP:0000107	Renal cyst	4	219	1.47	0.00048	BBS1,LAMA3,LAMB3,FLNB
Monarch	HP:0001939	Abnormality of metabolism/homeostasis	8	2167	0.78	0.00048	ITGB4,EDAR,CDH1,BBS1,LAMA3,LAMB3,FGFR1,FLNB
Monarch	HP:0000366	Abnormality of the nose	7	1469	0.89	0.00049	ITGB4,EDAR,CDH1,BBS1,KREMEN1,FGFR1,FLNB
Monarch	HP:0001510	Growth delay	7	1526	0.87	0.00061	ITGB4,BBS1,LAMA3,EVC2,LAMB3,FGFR1,FLNB
Monarch	HP:0000072	Hydroureter	3	69	1.85	0.00063	LAMA3,EVC2,LAMB3
Monarch	HP:0000966	Hypohidrosis	3	69	1.85	0.00063	WNT10A,EDAR,FGFR1
Monarch	HP:0012385	Camptodactyly	4	255	1.41	0.00081	ITGB4,LAMA3,LAMB3,FGFR1
Monarch	HP:0000069	Abnormality of the ureter	4	256	1.41	0.00082	ITGB4,LAMA3,EVC2,LAMB3
Monarch	HP:0002013	Vomiting	4	259	1.4	0.0008	ITGB4,LAMA3,LAMB3,F

						4	GFR1
Monarch	HP:0001780	Abnormality of toe	5	588	1.14	0.0009	ITGB4,BBS1,EVC2,FGFR1
						6	,FLNB
Monarch	HP:0001241	Capitate-hamate fusion	2	9	2.56	0.0011	EVC2,FLNB
Monarch	HP:0001615	Hoarse cry	2	9	2.56	0.0011	LAMA3,LAMB3
Monarch	HP:0002715	Abnormality of the immune system	7	1700	0.83	0.0011	ITGB4,EDAR,BBS1,LAMA 3,EVC2,LAMB3,FGFR1
Monarch	HP:0000707	Abnormality of the nervous system	9	3470	0.63	0.0013	ITGB4,WNT10A,CDH1,BB S1,LAMA3,EVC2,LAMB3, FGFR1,FLNB
Monarch	HP:0000706	Eruption failure	2	11	2.47	0.0014	FGFR1,FLNB
Monarch	HP:0001581	Recurrent skin infections	3	96	1.71	0.0014	ITGB4,LAMA3,LAMB3
Monarch	HP:0001818	Paronychia	2	11	2.47	0.0014	LAMA3,LAMB3
Monarch	HP:0011053	Agenesis of mandibular premolar	2	11	2.47	0.0014	WNT10A,FGFR1
Monarch	HP:0011056	Agenesis of first permanent molar tooth	2	11	2.47	0.0014	WNT10A,FGFR1
Monarch	HP:0012368	Flat face	3	94	1.72	0.0014	CDH1,FGFR1,FLNB
Monarch	HP:0000202	Oral cleft	5	666	1.09	0.0016	WNT10A,CDH1,EVC2,FG FR1,FLNB

Monarch	HP:0000481	Abnormal cornea morphology	5	675	1.08	0.0016	BBS1,LAMA3,LAMB3,FGFR1,FLNB
Monarch	HP:0001156	Brachydactyly	4	313	1.32	0.0016	BBS1,EVC2,FGFR1,FLNB
Monarch	HP:0001792	Small nail	3	103	1.68	0.0016	WNT10A,CDH1,EVC2
Monarch	HP:0004400	Abnormality of the pylorus	3	103	1.68	0.0016	ITGB4,LAMA3,LAMB3
Monarch	HP:0006336	Short dental root	2	12	2.43	0.0016	WNT10A,FGFR1
Monarch	HP:0012252	Abnormal respiratory system morphology	4	314	1.32	0.0016	LAMA3,LAMB3,FGFR1,FLNB
Monarch	HP:0000119	Abnormality of the genitourinary system	4	322	1.31	0.0017	ITGB4,WNT10A,CDH1,FGFR1
Monarch	HP:0000003	Multicystic kidney dysplasia	3	107	1.66	0.0018	BBS1,LAMA3,LAMB3
Monarch	HP:0001627	Abnormal heart morphology	6	1204	0.91	0.0018	BBS1,LAMA3,EVC2,LAMB3,FGFR1,FLNB
Monarch	HP:0006289	Agenesis of central incisor	2	13	2.4	0.0018	WNT10A,FGFR1
Monarch	HP:0006342	Peg-shaped maxillary lateral incisors	2	13	2.4	0.0018	WNT10A,FGFR1
Monarch	HP:0006344	Abnormality of primary	2	13	2.4	0.0018	WNT10A,FGFR1

		molar morphology					
Monarch	HP:0000690	Agenesis of maxillary lateral incisor	2	14	2.37	0.002	WNT10A,FGFR1
Monarch	HP:0001806	Onycholysis	2	14	2.37	0.002	WNT10A,LAMA3
Monarch	HP:0011078	Abnormality of canine	2	14	2.37	0.002	WNT10A,FGFR1
Monarch	HP:0011354	Generalized abnormality of skin	4	340	1.28	0.002	WNT10A,EDAR,EVC2,FGFR1
Monarch	HP:0100518	Dysuria	2	14	2.37	0.002	LAMA3,LAMB3
Monarch	HP:0004097	Deviation of finger	3	117	1.62	0.0022	BBS1,EVC2,FLNB
Monarch	HP:0004275	Duplication of hand bones	3	121	1.61	0.0024	EVC2,FGFR1,FLNB
Monarch	HP:0008873	Disproportionate short-limb short stature	3	122	1.6	0.0024	EVC2,FGFR1,FLNB
Monarch	HP:0011219	Short face	2	16	2.31	0.0024	WNT10A,FGFR1
Monarch	HP:0012472	Eclabion	3	122	1.6	0.0024	WNT10A,EDAR,FGFR1
Monarch	HP:0100336	Bilateral cleft lip	2	16	2.31	0.0024	CDH1,FGFR1
Monarch	HP:0001955	Unexplained fevers	2	17	2.28	0.0026	LAMA3,LAMB3
Monarch	HP:0002046	Heat intolerance	2	17	2.28	0.0026	ITGB4,EDAR
Monarch	HP:0031703	Abnormal ear morphology	6	1319	0.87	0.0027	ITGB4,CDH1,BBS1,EVC2,FGFR1,FLNB

Monarch	HP:0003508	Proportionate short stature	3	131	1.57	0.0029	EVC2,FGFR1,FLNB
Monarch	HP:0002780	Bronchomalacia	2	19	2.23	0.0031	FGFR1,FLNB
Monarch	HP:0000958	Dry skin	3	136	1.56	0.0032	WNT10A,EDAR,FGFR1
Monarch	HP:0001159	Syndactyly	3	138	1.55	0.0033	CDH1,BBS1,FGFR1
Monarch	HP:0000081	Duplicated collecting system	2	20	2.21	0.0034	LAMA3,LAMB3
Monarch	HP:0002043	Esophageal stricture	2	20	2.21	0.0034	LAMA3,LAMB3
Monarch	HP:0006323	Premature loss of primary teeth	2	21	2.19	0.0036	WNT10A,EDAR
Monarch	HP:0009767	Aplasia/Hypoplasia of the phalanges of the hand	3	144	1.53	0.0036	EVC2,FGFR1,FLNB
Monarch	HP:0006315	Solitary median maxillary central incisor	2	22	2.17	0.0039	EVC2,FGFR1
Monarch	HP:0000032	Abnormality of male external genitalia	5	852	0.98	0.004	ITGB4,BBS1,EVC2,FGFR1,FLNB
Monarch	HP:0001810	Dystrophic toenail	2	23	2.15	0.0041	ITGB4,WNT10A
Monarch	HP:0005048	Synostosis of carpal bones	2	23	2.15	0.0041	EVC2,FGFR1
Monarch	HP:0100490	Camptodactyly of finger	3	153	1.5	0.0041	ITGB4,LAMA3,LAMB3

Monarch	HP:0000586	Shallow orbits	2	24	2.13	0.0044	FGFR1,FLNB
Monarch	HP:0004395	Malnutrition	2	24	2.13	0.0044	LAMA3,LAMB3
Monarch	HP:0030084	Clinodactyly	4	442	1.17	0.0044	CDH1,EVC2,FGFR1,FLNB
Monarch	HP:0040069	Abnormal lower limb bone morphology	4	442	1.17	0.0044	BBS1,EVC2,FGFR1,FLNB
Monarch	HP:0002088	Abnormal lung morphology	5	894	0.96	0.0046	LAMA3,EVC2,LAMB3,FGFR1,FLNB
Monarch	HP:0008368	Tarsal synostosis	2	25	2.12	0.0046	FGFR1,FLNB
Monarch	HP:0012638	Abnormal nervous system physiology	8	3153	0.62	0.0046	ITGB4,WNT10A,BBS1,LAMA3,EVC2,LAMB3,FGFR1,FLNB
Monarch	HP:0009924	Aplasia/Hypoplasia involving the nose	2	26	2.1	0.0048	FGFR1,FLNB
Monarch	HP:0000016	Urinary retention	2	27	2.08	0.0051	LAMA3,LAMB3
Monarch	HP:0001769	Broad foot	2	27	2.08	0.0051	BBS1,FGFR1
Monarch	HP:0004348	Abnormality of bone mineral density	4	469	1.14	0.0052	ITGB4,LAMA3,LAMB3,FGFR1
Monarch	HP:0002032	Esophageal atresia	2	28	2.07	0.0053	ITGB4,FGFR1
Monarch	HP:0002143	Abnormality of the spinal cord	3	172	1.45	0.0053	ITGB4,FGFR1,FLNB

Monarch	HP:0011277	Abnormality of the urinary system physiology	5	931	0.94	0.0053	ITGB4,BBS1,LAMA3,LAMB3,FGFR1
Monarch	HP:0011805	Abnormal skeletal muscle morphology	6	1538	0.8	0.0053	ITGB4,BBS1,LAMA3,LAMB3,FGFR1,FLNB
Monarch	HP:0000684	Delayed eruption of teeth	3	174	1.45	0.0054	WNT10A,EVC2,FGFR1
Monarch	HP:0000705	Amelogenesis imperfecta	2	29	2.05	0.0056	LAMA3,LAMB3
Monarch	HP:0006000	Ureteral obstruction	2	29	2.05	0.0056	LAMA3,LAMB3
Monarch	HP:0009122	Aplasia/hypoplasia affecting bones of the axial skeleton	3	177	1.44	0.0056	EVC2,FGFR1,FLNB
Monarch	HP:0011123	Inflammatory abnormality of the skin	4	482	1.13	0.0056	ITGB4,EDAR,LAMA3,LAMB3
Monarch	HP:0000696	Delayed eruption of permanent teeth	2	30	2.04	0.0059	WNT10A,FGFR1
Monarch	HP:0002098	Respiratory distress	3	183	1.43	0.006	LAMA3,LAMB3,FGFR1
Monarch	HP:0012575	Abnormal nephron morphology	3	183	1.43	0.006	ITGB4,LAMA3,LAMB3
Monarch	HP:0008391	Dystrophic fingernails	2	31	2.02	0.0062	ITGB4,WNT10A
Monarch	HP:0009811	Abnormality of the	3	186	1.42	0.0062	EVC2,FGFR1,FLNB

		elbow					
Monarch	HP:0010307	Stridor	2	31	2.02	0.0062	LAMA3,LAMB3
Monarch	HP:0009381	Short finger	3	188	1.42	0.0064	EVC2,FGFR1,FLNB
Monarch	HP:0012649	Increased inflammatory response	5	984	0.92	0.0064	ITGB4,EDAR,LAMA3,LAMB3,FGFR1
Monarch	HP:0005280	Depressed nasal bridge	4	511	1.11	0.0066	EDAR,KREMEN1,FGFR1,FLNB
Monarch	HP:0009826	Limb undergrowth	3	191	1.41	0.0066	EVC2,FGFR1,FLNB
Monarch	HP:0001626	Abnormality of the cardiovascular system	7	2435	0.67	0.0068	WNT10A,BBS1,LAMA3,EVC2,LAMB3,FGFR1,FLNB
Monarch	HP:0002086	Abnormality of the respiratory system	6	1638	0.78	0.0068	BBS1,LAMA3,EVC2,LAMB3,FGFR1,FLNB
Monarch	HP:0009803	Short phalanx of finger	3	195	1.4	0.0069	EVC2,FGFR1,FLNB
Monarch	HP:0000932	Abnormal posterior cranial fossa morphology	3	197	1.39	0.007	EVC2,FGFR1,FLNB
Monarch	HP:0002778	Abnormal tracheal morphology	2	35	1.97	0.0074	FGFR1,FLNB
Monarch	HP:0000356	Abnormality of the outer ear	5	1032	0.9	0.0077	ITGB4,BBS1,EVC2,FGFR1,FLNB
Monarch	HP:0002763	Abnormal cartilage	2	36	1.96	0.0077	FGFR1,FLNB

		morphology					
Monarch	HP:0005216	Impaired mastication	2	37	1.95	0.0081	WNT10A,FGFR1
Monarch	HP:0009738	Abnormal antihelix morphology	2	37	1.95	0.0081	EVC2,FGFR1
Monarch	EFO:0000405	Digestive system disease	3	211	1.37	0.0084	LAMA3,KREMEN1,LAMB3
Monarch	HP:0002087	Abnormality of the upper respiratory tract	3	215	1.36	0.0088	LAMA3,LAMB3,FGFR1
Monarch	HP:0002107	Pneumothorax	2	40	1.91	0.0092	LAMA3,LAMB3
Monarch	HP:0002093	Respiratory insufficiency	4	578	1.05	0.0099	LAMA3,LAMB3,FGFR1,FLNB
Monarch	EFO:0003765	Sign or symptom	4	580	1.05	0.01	ITGB4,LAMA3,LAMB3,FGFR1
Monarch	HP:0006487	Bowing of the long bones	3	226	1.34	0.01	EVC2,FGFR1,FLNB
Monarch	HP:0003026	Short long bone	3	229	1.33	0.0104	EVC2,FGFR1,FLNB
Monarch	HP:0000939	Osteoporosis	3	230	1.33	0.0105	LAMA3,LAMB3,FGFR1
Monarch	HP:0001800	Hypoplastic toenails	2	46	1.85	0.0118	EVC2,FGFR1
Monarch	HP:0000873	Diabetes insipidus	2	47	1.84	0.0122	BBS1,FGFR1
Monarch	HP:0000028	Cryptorchidism	4	617	1.02	0.0124	BBS1,EVC2,FGFR1,FLNB

Monarch	HP:0002090	Pneumonia	3	245	1.3	0.0124	LAMA3,LAMB3,FGFR1
Monarch	HP:0011100	Intestinal atresia	2	48	1.83	0.0126	ITGB4,FGFR1
Monarch	HP:0002991	Abnormality of fibula morphology	2	49	1.82	0.013	FGFR1,FLNB
Monarch	HP:0000946	Hypoplastic ilia	2	50	1.81	0.0134	EVC2,FLNB
Monarch	EFO:0007825	Balding measurement	3	256	1.28	0.0138	WNT10A,EDAR,KREMEN1
Monarch	HP:0000384	Preauricular skin tag	2	51	1.81	0.0139	FGFR1,FLNB
Monarch	HP:0000656	Ectropion	2	51	1.81	0.0139	ITGB4,CDH1
Monarch	HP:0100615	Ovarian neoplasm	2	51	1.81	0.0139	WNT10A,CDH1
Monarch	HP:0000316	Hypertelorism	4	649	1	0.0143	CDH1,KREMEN1,FGFR1,FLNB
Monarch	HP:0003839	Abnormality of upper limb epiphysis morphology	2	52	1.8	0.0143	EVC2,FLNB
Monarch	HP:0010743	Short metatarsal	2	52	1.8	0.0143	FGFR1,FLNB
Monarch	HP:0003063	Abnormality of the humerus	2	54	1.78	0.0151	FGFR1,FLNB
Monarch	HP:0001608	Abnormality of the voice	3	269	1.26	0.0154	LAMA3,LAMB3,FGFR1
Monarch	HP:0000535	Sparse and thin eyebrow	2	55	1.77	0.0155	WNT10A,EDAR

Monarch	HP:0000929	Abnormal skull morphology	6	1955	0.7	0.0155	ITGB4,EDAR,BBS1,EVC2, FGFR1,FLNB
Monarch	HP:0009466	Radial deviation of finger	2	55	1.77	0.0155	BBS1,FLNB
Monarch	HP:0001384	Abnormal hip joint morphology	3	274	1.25	0.0159	EVC2,FGFR1,FLNB
Monarch	HP:0002242	Abnormal intestine morphology	4	673	0.99	0.0159	ITGB4,BBS1,FGFR1,FLNB
Monarch	HP:0003111	Abnormal blood ion concentration	3	277	1.25	0.0163	LAMA3,LAMB3,FGFR1
Monarch	HP:0002019	Constipation	3	279	1.24	0.0166	LAMA3,LAMB3,FGFR1
Monarch	HP:0002750	Delayed skeletal maturation	3	279	1.24	0.0166	EVC2,FGFR1,FLNB
Monarch	HP:0000653	Sparse eyelashes	2	58	1.75	0.0167	EDAR,KREMEN1
Monarch	HP:0008905	Rhizomelia	2	58	1.75	0.0167	FGFR1,FLNB
Monarch	HP:0003170	Abnormal acetabulum morphology	2	59	1.74	0.0172	EVC2,FLNB
Monarch	HP:0001367	Abnormal joint morphology	4	694	0.97	0.0174	ITGB4,EVC2,FGFR1,FLNB
Monarch	HP:0010301	Spinal dysraphism	2	61	1.73	0.018	ITGB4,FGFR1

Monarch	HP:0010720	Abnormal hair pattern	3	289	1.23	0.018	ITGB4,CDH1,KREMEN1
Monarch	HP:0004209	Clinodactyly of the 5th finger	3	291	1.23	0.0182	EVC2,FGFR1,FLNB
Monarch	HP:0001211	Abnormal fingertip morphology	2	63	1.71	0.019	LAMA3,LAMB3
Monarch	HP:0001539	Omphalocele	2	64	1.71	0.0195	FGFR1,FLNB
Monarch	HP:0002648	Abnormality of calvarial morphology	3	299	1.21	0.0195	ITGB4,FGFR1,FLNB
Monarch	HP:0001631	Atrial septal defect	3	307	1.2	0.0208	EVC2,FGFR1,FLNB
Monarch	HP:0002213	Fine hair	2	69	1.67	0.0222	WNT10A,EDAR
Monarch	HP:0001662	Bradycardia	2	70	1.67	0.0226	LAMA3,LAMB3
Monarch	HP:0004386	Gastrointestinal inflammation	2	71	1.66	0.0232	LAMA3,LAMB3
Monarch	HP:0000430	Underdeveloped nasal alae	2	72	1.66	0.0237	ITGB4,FLNB
Monarch	HP:0008724	Hypoplasia of the ovary	2	72	1.66	0.0237	BBS1,FGFR1
Monarch	HP:0000470	Short neck	3	325	1.18	0.0238	BBS1,FGFR1,FLNB
Monarch	HP:0002007	Frontal bossing	3	330	1.17	0.0247	EDAR,FGFR1,FLNB
Monarch	HP:0000795	Abnormality of the urethra	3	335	1.16	0.0256	ITGB4,EVC2,FGFR1

Monarch	HP:0001883	Talipes	3	350	1.15	0.0286	EVC2,FGFR1,FLNB
Monarch	HP:0006501	Aplasia/Hypoplasia of the radius	2	81	1.6	0.0286	FGFR1,FLNB
Monarch	HP:0009882	Short distal phalanx of finger	2	85	1.58	0.0312	EVC2,FLNB
Monarch	HP:0000687	Widely spaced teeth	2	86	1.58	0.0317	WNT10A,FGFR1
Monarch	HP:0004323	Abnormality of body weight	5	1512	0.73	0.0342	BBS1,LAMA3,EVC2,LAMB3,FGFR1
Monarch	HP:0001629	Ventricular septal defect	3	377	1.11	0.0346	EVC2,FGFR1,FLNB
Monarch	HP:0000678	Dental crowding	2	92	1.55	0.0357	BBS1,FGFR1
Monarch	HP:0100806	Sepsis	2	92	1.55	0.0357	LAMA3,LAMB3
Monarch	HP:0000969	Edema	3	386	1.1	0.0362	LAMA3,LAMB3,FLNB
Monarch	HP:0004279	Short palm	2	94	1.54	0.0366	EVC2,FGFR1
Monarch	HP:0011304	Broad thumb	2	94	1.54	0.0366	FGFR1,FLNB
Monarch	HP:0002983	Micromelia	2	96	1.53	0.0378	EVC2,FLNB
Monarch	HP:0001162	Postaxial hand polydactyly	2	97	1.53	0.0383	BBS1,EVC2
Monarch	HP:0003510	Severe short stature	2	97	1.53	0.0383	FGFR1,FLNB
Monarch	HP:0000010	Recurrent urinary tract infections	2	98	1.52	0.0389	LAMA3,LAMB3

Monarch	HP:0030956	Abnormality of cardiovascular system electrophysiology	3	403	1.08	0.0399	LAMA3,LAMB3,FGFR1
Monarch	HP:0001711	Abnormal left ventricle morphology	2	100	1.51	0.0403	BBS1,FGFR1
Monarch	HP:0002021	Pyloric stenosis	2	100	1.51	0.0403	LAMA3,LAMB3
Monarch	HP:0200042	Skin ulcer	2	100	1.51	0.0403	ITGB4,LAMA3
Monarch	HP:0000453	Choanal atresia	2	101	1.51	0.0408	CDH1,FGFR1
Monarch	HP:0001161	Hand polydactyly	2	102	1.5	0.0414	EVC2,FGFR1
Monarch	HP:0000689	Dental malocclusion	2	103	1.5	0.0421	WNT10A,FGFR1
Monarch	HP:0001944	Dehydration	2	104	1.5	0.0427	LAMA3,LAMB3
Monarch	HP:0000327	Hypoplasia of the maxilla	2	105	1.49	0.0434	FGFR1,FLNB
Monarch	HP:0000457	Depressed nasal ridge	2	105	1.49	0.0434	EDAR,FGFR1
Monarch	HP:0000534	Abnormal eyebrow morphology	3	419	1.07	0.0434	WNT10A,KREMEN1,FGFR1
Monarch	HP:0001773	Short foot	2	105	1.49	0.0434	BBS1,FGFR1
Monarch	HP:0000601	Hypotelorism	2	106	1.49	0.0436	EVC2,FGFR1
Monarch	HP:0000824	Decreased response to growth hormone	2	107	1.48	0.0442	FGFR1,FLNB

		stimulation test					
Monarch	HP:0001903	Anemia	3	426	1.06	0.0448	ITGB4,LAMA3,LAMB3
Monarch	HP:0001508	Failure to thrive	4	950	0.84	0.0455	LAMA3,EVC2,LAMB3,FGFR1
Monarch	HP:0002084	Encephalocele	2	109	1.48	0.0455	FGFR1,FLNB
Monarch	HP:0000926	Platyspondyly	2	111	1.47	0.0465	FGFR1,FLNB
Monarch	HP:0010049	Short metacarpal	2	113	1.46	0.0479	FGFR1,FLNB
Monarch	HP:0000774	Narrow chest	2	114	1.46	0.0486	EVC2,FLNB
Monarch	HP:0000177	Abnormality of upper lip	2	115	1.45	0.0492	EDAR,FGFR1
Monarch	HP:0001999	Abnormal facial shape	4	978	0.82	0.0492	WNT10A,CDH1,FGFR1,FLNB
DISEASES	DOID:3209	Junctional epidermolysis bullosa	3	8	2.79	0.00013	ITGB4,LAMA3,LAMB3
DISEASES	DOID:0050739	Autosomal genetic disease	9	2323	0.8	0.00082	ITGB4,EDAR,CDH1,BBS1,LAMA3,EVC2,LAMB3,FGFR1,FLNB
DISEASES	DOID:225	Syndrome	7	1059	1.03	0.00082	WNT10A,EDAR,CDH1,BBS1,EVC2,FGFR1,FLNB
DISEASES	DOID:0060737	Junctional epidermolysis bullosa Herlitz type	2	3	3.04	0.0025	LAMA3,LAMB3

DISEASES	DOID:0050737	Autosomal recessive disease	7	1422	0.9	0.0033	ITGB4,EDAR,BBS1,LAMA3,EVC2,LAMB3,FLNB
DISEASES	DOID:37	Skin disease	5	481	1.23	0.0033	ITGB4,WNT10A,EDAR,LAMA3,LAMB3
DISEASES	DOID:0050591	Tooth agenesis	2	7	2.67	0.0045	WNT10A,EDAR
DISEASES	DOID:1091	Tooth disease	3	81	1.78	0.0052	WNT10A,EDAR,LAMB3
DISEASES	DOID:14793	Hypohidrotic ectodermal dysplasia	2	8	2.61	0.0052	WNT10A,EDAR
DISEASES	DOID:4	Disease	11	5921	0.48	0.0052	ITGB4,WNT10A,EDAR,CDH1,BBS1,LAMA3,EVC2,SMOC2,LAMB3,FGFR1,FLNB
DISEASES	DOID:7	Disease of anatomical entity	10	4452	0.56	0.0052	ITGB4,WNT10A,EDAR,CDH1,LAMA3,EVC2,SMOC2,LAMB3,FGFR1,FLNB
DISEASES	DOID:77	Gastrointestinal system disease	4	510	1.11	0.0477	WNT10A,EDAR,CDH1,LAMB3
COMPARTMENTS	GOCC:0005610	laminin-5 complex	2	7	2.67	0.0286	LAMA3,LAMB3

UniProt Keywords	KW-0038	Ectodermal dysplasia	5	49	2.22	6.96E-08	WNT10A,EDAR,CDH1,KREMEN1,EVC2
UniProt Keywords	KW-0263	Epidermolysis bullosa	3	16	2.49	0.0000573	ITGB4,LAMA3,LAMB3
UniProt Keywords	KW-9995	Disease	11	3996	0.65	0.0000574	ITGB4,WNT10A,EDAR,CDH1,BBS1,LAMA3,KREMEN1,EVC2,LAMB3,FGFR1,FLNB
UniProt Keywords	KW-0225	Disease mutation	10	3145	0.71	0.0000949	ITGB4,WNT10A,EDAR,CDH1,BBS1,KREMEN1,EVC2,LAMB3,FGFR1,FLNB
UniProt Keywords	KW-0732	Signal	10	3233	0.7	0.0000991	ITGB4,WNT10A,EDAR,CDH1,LAMA3,KREMEN1,EVC2,SMOC2,LAMB3,FGFR1
UniProt Keywords	KW-0084	Basement membrane	3	39	2.1	0.00022	LAMA3,SMOC2,LAMB3
UniProt Keywords	KW-0325	Glycoprotein	10	4349	0.57	0.0012	ITGB4,WNT10A,EDAR,CDH1,LAMA3,KREMEN1,EVC2,SMOC2,LAMB3,FGF

							R1
UniProt Keywords	KW-0272	Extracellular matrix	4	265	1.39	0.0013	WNT10A,LAMA3,SMOC2, LAMB3
UniProt Keywords	KW-1015	Disulfide bond	9	3304	0.65	0.0013	ITGB4,WNT10A,EDAR,CDH1,LAMA3,KREMEN1,SMOC2,LAMB3,FGFR1
UniProt Keywords	KW-0130	Cell adhesion	4	474	1.14	0.0095	ITGB4,CDH1,LAMA3,LAMB3
UniProt Keywords	KW-0242	Dwarfism	3	170	1.46	0.0095	EVC2,FGFR1,FLNB
UniProt Keywords	KW-0424	Laminin EGF-like domain	2	30	2.04	0.0095	LAMA3,LAMB3
SMART	SM00136	Laminin N-terminal domain (domain VI)	2	15	2.34	0.0338	LAMA3,LAMB3

IV. DISCUSSION

To the best of our knowledge, this is the first study to reveal the underlying genetic variants in a specific phenotype of hypodontia using WES and bioinformatics analyses. Bilateral mandibular second premolar hypodontia was selected as a phenotype for the following reasons. First, because rodents do not have premolars, it is difficult to unveil the pathogenesis of premolar agenesis using experiments. Second, maxillary and mandibular teeth are reported to be developed by different genetic programs (Ferguson et al., 2000; Cobourne and Sharpe, 2003), and extrinsic factors could be involved in unilateral hypodontia (De Coster et al., 2009).

No significant variants were found in the case-control group comparisons. It was unable to identify the specific variant that may affect the agenesis of the mandibular second premolars. This might be due to the small sample, but considering the genetic heterogeneity of tooth agenesis, it is probable that a single critical variant for the specific phenotype of the disease may not exist. Instead, considering oligodontia is a rare disease (almost 0.1% prevalence) and a severe form of tooth agenesis, it is interesting that the same variants reported to cause oligodontia were also found in these hypodontia patients. Missense variants (c.1138A>C) and (c.511C>T) in *EDAR* and *WNT10A*, respectively, have been previously reported in oligodontia patients. The results of recent WES investigations in tooth agenesis proposed the idea of mutational load with oligogenic inheritance and multilocus variation models (Dinckan et al., 2018; Andersson et al., 2020; Posey et al., 2017). This variable expressivity reinforces the theory that variations in

several genes, working either alone or in concert with other genes, may determine the severity of NSTA (Williams and Letra, 2018; Dreesen et al., 2014; Arte et al., 2013). It is still controversial and the reason is yet to be understood, but females were found to have a higher prevalence of hypodontia than males from the systematic reviews (Khalaf et al., 2014; Polder et al., 2004). Case group was mainly composed of males, and this imbalance in gender may have affected the outcome as well.

Notably, *LAMA3, LAMB3*, and *ITGB4* are involved in junctional epidermolysis bullosa (Fine et al., 2008). These proteins are involved in basement membrane-mediated cell adhesion and are critical for proper tooth development (Aberdam et al., 1994; Asaka et al., 2009). Key terms from functional enrichment analysis included “cell junction,” “extracellular matrix,” “basement membrane,” and “laminin.” Moreover, the hypodontia group had approximately 2-fold as many mutated variants in all four genes related to these key terms as the control group. Basement membranes are specialized extracellular matrices composed primarily of different types of laminin, type IV collagen, perlecan, and nidogen (Timpl, 1996). During early development, they act as a physical barrier and regulatory structure for reciprocal signaling between the oral epithelium and mesenchyme layers. These basement membrane matrices regulate proliferation, polarity, and adhesion, as well as the size and morphology of tooth germs (Yoshizaki and Yamada, 2013). Early in the development of the mouse embryo, type IV collagen and laminin molecules are evenly distributed in the dental basement membranes, and they vanish as the membranes are broken down by enzymes (Lesot et al., 1981; Kjoelby et al., 1994). The basement membrane also

becomes discontinuous and vanishes at the beginning of the dentin mineralization process (Kawashima and Okiji, 2016). Considering the critical roles of these key terms and the importance of fine balancing between complex networks of signaling pathways during the early odontogenic process, enriched terms may be involved in the pathogenesis of tooth agenesis.

Pathway-related enriched terms included “PI3K-Akt signaling pathway” and “α6β4 signaling pathway”. Several pathways act early in development to determine the differential location, identity, shape, and size of teeth. Even if no specific gene is responsible for each tooth shape, these enriched terms are more likely to be related to molar regions rather than incisor regions. BMP4 and FGF8 are the first molecular signals that initiate differential tooth morphogenesis (Laugel-Haushalter et al., 2013). Fgf8 is expressed in the proximal (presumptive molar region) oral epithelium and promotes Barx1 expression, whereas Bmp4 is initially expressed in the distal (presumptive incisor) epithelium (Tucker et al., 1998). The PI3K-Akt signaling pathway has been associated with cell growth, cell cycle regulation, and cell survival. Recently, it has been proposed to function as an intracellular pathway for transducing FGF8 signals into nuclei in dental mesenchyme, preventing cell apoptosis (Lin et al., 2022). Integrin α6β4 is thought to mediate laminin-10/11-induced cell spreading and filopodia formation of the dental epithelium, implying that these interactions play an important role in determining the size and shape of tooth germs. The interaction between laminin-10/11 and integrin α6β4 is also thought to be required for PI3K/Akt pathway activation in the dental epithelium (Fukumoto et al., 2006). Furthermore, integrin α6β4 stimulates Rac1 and RhoA activation, which modulate cuspal shape decisions by

coordinating adhesion junctions, actin distribution, and fibronectin localization to trigger inner dental epithelium invagination (Stewart and O'Connor, 2015; Li et al., 2016).

PITX1, which encodes a novel bicoid-related family of homeoproteins that is differentially expressed between the upper and lower molars, is one of the prime candidate genes for controlling maxillary/mandibular tooth identity (Laugel-Haushalter et al., 2013). *Pitx1* is expressed in the proximal mesenchyme of the developing mandible, hindlimb, oral epithelium, developing teeth, and pituitary gland. Inactivation of the *Pitx1* gene in mice impacts mandibular tooth morphogenesis (Mitsiadis and Drouin, 2008). Top-ranked terms from functional analyses of *Pitx1*-dependent genes have included the extracellular matrix during hindlimb development, and differentially expressed gene analysis between control and PITX1-overexpressing osteosarcoma genes also categorized the extracellular matrix and PI3K/Akt in the top 20 KEGG pathways (Nemec et al., 2017; Wang et al., 2018; Zhang et al., 2023).

Laminin α3 (the LAMA3 protein) is an element of laminin-5 (α3β3γ2) that regulates epithelial cell anchoring and motility via the integrins α6β4 and α3β1, respectively (Fukumoto and Yamada, 2005). In an animal study, Lama3-targeted knockout mice showed abnormalities in ameloblast differentiation (Ryan et al., 1999). *LAMA3* mutations have also been previously reported in non-syndromic hypodontia patients (Dinckan et al., 2018). This study also identified a stop-gain variant (c.4750C>T) in *LAMA3* from the participants. To our knowledge, this is the third report of the *LAMA3* mutation in non-syndromic tooth agenesis. The exact mechanism by which *LAMA3* mutations lead to hypodontia is not fully understood, but it is thought to be related

to the role of laminin-5 in tooth bud development and the attachment of teeth to the surrounding tissues. Further, other candidate genes sharing similar functions and enriched terms to *LAMA3* may also have a similar role in mandibular premolar hypodontia.

In a genome-wide association study (GWAS) of NSTA, including hypodontia and oligodontia, the rs917412-T variant was reported to be associated with the agenesis of mandibular second premolars (Jonsson et al., 2018). However, none of the samples had this variant possibly because of differences in allele frequency between different ethnicities. The allele frequency of rs917412-T is 0.0007704 in East Asians, which is significantly lower than the overall allele frequency of 0.2054 in the gnomAD database.

There are some limitations to this pilot study. First, the sample size was small. Therefore, this study attempted to overcome this shortcoming by specifying the phenotype of the disease. Second, the development of teeth is very complex, and interpretation of the impact variants have on the disease is challenging. Simple case-control comparisons may not be able to explain the pathogenicity of tooth agenesis. Pathways and signaling interactions that intersect and interplay during tooth development may be the key to the genotype–phenotype correlation. Third, this study investigated genetic variants of unrelated individuals that share the same disease phenotype. Therefore, there is a need for segregation analysis to further verify candidate gene variant impact. Lastly, hypodontia may arise due to the mutations in non-coding regions such as gene regulatory domains or epigenetic alterations. A recent study which investigated the role of DNA methylation in NSTA reported that there were significant differences in the methylation level of the whole

genome between the hypodontia and normal groups (Wang et al., 2016). The application of whole-genome sequencing analysis also may help further elucidate the pathogenesis of hypodontia.

V. CONCLUSION

This study specified a phenotype for non-syndromic hypodontia as agenesis of the bilateral mandibular second premolars in a Korean population and attempted to reveal the pathogenesis of the disease using WES and bioinformatics. This study found some known oligodontia gene variants in hypodontia patients, strengthening the possibility of additive effects in other genes and oligogenic inheritance in tooth agenesis. Furthermore, this study identified some functionally enriched terms important to mandibular molar development and explored the possible common features of the etiology of mandibular premolar hypodontia. This study is a viable preliminary attempt to reveal the pathogenesis of tooth agenesis and may help design future tooth agenesis studies. Since there are various types of teeth, further studies with other phenotypes and larger populations are required to compare and understand the disease in the future.

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ABSTRACT (KOREAN)

전장엑솜분석을 이용한 하악 소구치 부분무치증의 원인 유전자 변이 분석

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부분무치증(hypodontia)을 가지는 환자들에게서 유전자형-표현형 상관관계를 파악하는 것은 질환의 병인을 이해하기 위해서 중요하지만, 아직까지 이에 관한 연구는 부족한 실정이다. 이에 따라 본 연구는 전장엑솜분석 및 다양한 생물정보학분석을 이용하여 한국인에게서 양측성으로 하악 제2소구치가 결손된 특정한 표현형을 가지는 non-syndromic hypodontia와 연관된 유전적 변이형을 발견하는 것을 목적으로 하였다. 서로 연관이 없는 독립된 20명의 참가자들로부터 전장엑솜분석을 시행하였으며, 기존에 연구를 통해 알려진 112개의 tooth agenesis 관련 유전자들로부터 변이들의 필터링과 선별과정을 통하여 잠재적인 후보 변이들을 선별하였다. 최종적으로 12개의 후

보 유전자들로부터 13개의 후보 변이들을 발견하였으며 이 중에서는 *LAMA3* 유전자의 stop-gain 변이(c.4750C>T)도 포함되었다. 선별된 유전자들의 기능농축분석을 통하여 하악 제2소구치 hypodontia 후보 유전자들의 단백질-단백질 상호작용 네트워크에서 치아 발생과 관련된 여러 기능 및 전달경로들이 강조된 것을 확인하였다. 또한 hypodontia 참가자군은 100명의 건강한 대조군 집단들과 비교하였을 때 기능농축분석에서 확인된 주요 기능들과 연관된 *CDH1, ITGB4, LAMA3, LAMB3*의 4개의 유전자에서 모두 약 2배 더 많은 변이를 가졌다. 마지막으로 기능농축분석을 통해 강조된 기능 및 경로들이 어떻게 하악 제2소구치의 선천적 결손과 연관될 수 있는지에 대해서도 논의하였다. 본 연구의 hypodontia 참가자들에게서 기존에 oligodontia 환자들에게서 보고된 변이들을 발견하였으며, 이번 결과로 tooth agenesis의 발생 정도가 여러 유전자들과의 협력효과를 통해 더 커질 수 있다는 가능성을 높였다. 이번 연구는 tooth agenesis의 병인을 밝히기 위한 좋은 예비 연구 및 후속 연구를 위한 배경이 될 수 있을 것으로 생각된다.

핵심되는 단어: 부분무치증; 유전자형-표현형 상관관계; 유전자연관연구; 생물정보학; 한국인 집단